REMARKS

Claims 1-4, 6-15, 18, 19, 37, 38, 40, 41, 50, 52-57 and 61-64 were pending. No claims are added and no claims are canceled herein. Thus, after entry of this amendment, **claims 1-4, 6-15, 18, 19, 37, 38, 40, 41, 50, 52-57 and 61-64 will still be pending**. Of these, claims 1-4, 6-15, 18, 19 and 50 are withdrawn. Claims 37, 38, 40, 41, 50, 52-57 and 61-64 are under consideration.

Claims 61-63 are amended to correct minor typographical errors or for clarity. No new matter has been introduced by these amendments.

CLAIM OBJECTION

Claim 63 is objected to for the recitation of "comprises and increase." Claim 63 is amended herein to recite "comprises increasing," rendering the objection moot.

REJECTION UNDER 35 U.S.C. § 103(a)

Claims 37, 38, 40, 41, 50, 52-57 and 61-64 are rejected under 35 U.S.C. § 103(a) as allegedly obvious over Ivins *et al.* (*Eur. J. Epidemiol.* 4(1):12-19, 1988), in view of Verthelyi *et al.* (*J. Immunol.* 168:1659-1663, 2002) and Jones *et al.* (*Vaccine* 17:3065-3071, 1999). The Office alleges that Ivins *et al.* teach the Anthrax Vaccine Adsorbed (AVA) vaccine, and that adjuvants containing alum or aluminum hydroxide were suboptimal. Verthelyi *et al.* is described as teaching the use of CpG oligonucleotides in primates as vaccine adjuvants, and Jones *et al.* is alleged to teach a CpG oligonucleotide having the nucleotide sequence of SEQ ID NO: 200. The Office concludes it would have been obvious to one of ordinary skill in the art to use the adjuvant of Jones *et al.* with the vaccine of Ivins *et al.*, and one would have been motivated to do so based on the teaching of Verthelyi *et al.* that CpG oligonucleotides can be used as adjuvants in primates. Applicants traverse this rejection.

Unpredictability

Based on the teachings of the prior art, it would not have been predictable that a CpG oligonucleotide comprising the nucleotide sequence of SEQ ID NO: 200 would enhance the immunogenicity of a vaccine against *Bacillus anthracis*. Although Jones *et al.* teaches the use of an oligonucleotide having the nucleotide sequence of SEQ ID NO: 200 as an adjuvant for a

vaccine against malaria, a disease caused by a protozoan parasite (*Plasmodium falciparum*), one would not have been able to predict that the same oligonucleotide would enhance the immunogenicity of a vaccine against *Bacillus anthracis*, a bacterial pathogen. As discussed in Su *et al.* (*Infect. Immun.* 71(9):5178-5187, 2003, a copy of which is provided herewith), it is not predictable that adjuvants that are effective with other types of vaccines are able to enhance an immune response against intracellular pathogens, including *Plasmodium* species. Eliciting protective immunity against intracellular pathogens, such as *Plasmodium falciparum*, is complex and requires both cellular and humoral immune responses. As taught by Applicants and the prior art, protective immune responses against *Bacillus anthracis* require production of neutralizing antibodies. Thus, it would not have been predictable that an adjuvant for a vaccine against an intracellular pathogen would be successful in enhancing an immune response when used in combination with a vaccine against a bacterial pathogen. Furthermore, Threadgill *et al.* (*Vaccine* 16(1):76-82, 1998, a copy of which is provided herewith), published before the priority date of the instant application, teach that administration of CpG oligonucleotide in combination with a bacterial (*Pseudomonas aeruginosa*) vaccine, actually diminishes the bacteria-specific antibody response. Given the teachings of Threadgill *et al.*, one of skill in the art would not have predicted that a CpG oligonucleotide would enhance the immunogenicity of a vaccine against a bacterial pathogen.

In addition, it would not have been predictable that a CpG oligonucleotide comprising the nucleotide sequence of SEQ ID NO: 200 would be an effective adjuvant for a complex, multi-component vaccine such as AVA. Jones *et al.* teach the use of CpG 7909 in combination with a simple peptide vaccine (PADRE 45). One of skill in the art would recognize that the immune response elicited in response to a simple peptide vaccine differs from the immune response following immunization with a complex vaccine. Accordingly, based on the combination of cited art, it would not have been predictable that an adjuvant effective for a peptide vaccine would also enhance the immunogenicity of a complex, multi-component vaccine such as AVA.

Prior art teaches away

The Office cites Verthelyi *et al.* as teaching the use of CpG oligonucleotides as vaccine adjuvants in primates. The authors tested both K-type and D-type CpG oligonucleotides in combination with a *Leishmania* vaccine. Verthelyi *et al.* teach (see page 1659) that K-type oligonucleotides "have phosphorothioate backbones, encode multiple TCGTT and/or TCGTA

motifs (CpG motif is underlined), trigger the maturation of plasmacytoid DC, and stimulate the production of IgM and IL-6." In contrast, D-type oligonucleotides "have mixed phosphodiester/phosphorothioate backbones and contain a single hexameric purine/pyrimidine/CG/purine/pyrimidine motif flanked by self-complementary bases that form a stem-loop structure capped at the 3' end by a poly G tail." CpG 7909 (SEQ ID NO: 200) is a K-type oligonucleotide. Verthelyi *et al.* teach that D-type oligonucleotides were superior to K-type oligonucleotides in enhancing the immune response following vaccination in primates. For example, Figure 5 shows the results of a challenge experiment in which macaques were vaccinated with HKLV (a heat killed *Leishmania* vaccine) and alum alone, or in combination with either D-type CpG oligonucleotide or K-type CpG oligonucleotide as adjuvant. Following challenge with *Leishmania major* metacyclic promastigotes, animals vaccinated with HKLV-alum developed typical cutaneous lesions with a peak surface area of approximately 300 mm^2 26 days after challenge. Addition of K-type oligonucleotide resulted in lesions of a similar size. However, animals that received the combination of HKLV-alum and D-type oligonucleotide exhibited significantly smaller lesions (about 80 mm^2), which is consistent with reduced parasite burden (see page 1661). Thus, one of skill in the art, based on the teachings of Verthelyi *et al.*, would have concluded that D-type oligonucleotides, and not K-type oligonucleotides, efficiently enhance immunogenicity of a complex vaccine in primates. Accordingly, Verthelyi *et al.* teaches away from the use of a K-type oligonucleotide (such as an oligonucleotide having the nucleotide sequence of SEQ ID NO: 200) and is therefore improperly combined with Ivins *et al.* and Jones *et al.*

Unexpectedly superior results

Administration of an oligonucleotide comprising the nucleotide sequence of SEQ ID NO: 200 in combination with a vaccine against *Bacillus anthracis* (the AVA vaccine) results in unexpectedly superior results compared with vaccine alone. As demonstrated in Example 4 of the application, following challenge with anthrax spores, mice passively immunized with non-human primate (NHP) serum obtained from rhesus macaques administered AVA in combination with CpG 7909 (SEQ ID NO: 200) had significantly increased survival relative to mice passively immunized with serum obtained from animals administered AVA alone (see Table VI). Passive immunization with serum from NHPs vaccinated with AVA and CpG 7909 (SEQ ID NO: 200) increased survival in mice in two separate experiments, using immune serum from either Day 11

or Day 16. For example, Day 11 serum from AVA plus CpG 7909 (SEQ ID NO: 200) vaccinated animals resulted in 60% survival of anthrax-challenged mice, relative to 10% survival following immunization with AVA only serum.

A post-priority date manuscript published by the inventors also provides evidence of unexpectedly superior results. Klinman *et al.* (*Vaccine* 22:2881-2886, 2004, a copy of which is provided herewith) describe experiments with rhesus macaques administered either AVA alone or AVA in combination with ODN 7909 (SEQ ID NO: 200). Klinman *et al.* report that AVA + ODN 7909 triggers an IgG anti-protective antigen (PA) response that is greater that AVA alone: "ODN 7909 plus AVA was significantly more immunogenic than AVA alone ($P < 0.05$), generating a >3-fold higher IgG anti-PA response over the first month ($P < 0.01$)" (page 2883, second column). Also of significant note is the finding that AVA in combination with ODN 7909 elicited a greater IgG anti-PA titer than AVA in combination with a mixture of three different CpG oligonucleotides (see Figure 3). Klinman *et al.* further report that "Macaques immunized with AVA + ODN 7909 had on average a 17-fold higher toxin neutralizing titer than those immunized with AVA alone" (emphasis added; page 2884, second column). As noted by Klinman *et al.*, stimulating neutralizing antibody against PA is critical for conferring protection against anthrax: "The induction of IgG anti-PA Abs is the most relevant measure of vaccine immunogenicity, since these Abs confer protection against infection" (page 2885, first column).

Klinman *et al.* further test the ability of neutralizing antibodies elicited in response to AVA or AVA + ODN 7909 to confer passive protection to mice. Mice that received pre-immune serum or serum from AVA vaccinated macaques rapidly succumbed to *Bacillus anthracis* challenge. In contrast, serum from macaques vaccinated with AVA + ODN 7909 protected approximately 50% of mice following challenge (see Figure 4). Klinman *et al.* summarize their findings by stating, "The combination of CpG ODN plus AVA triggered a faster, higher avidity, and higher-titered immune response than vaccine alone, resulting in a significant improvement in protective immunity against anthrax" (page 2885, first column). Thus, vaccination with AVA in combination with CpG 7909 (SEQ ID NO: 200) results in an unexpectedly superior immune response against *Bacillus anthracis*.

Administration of a CpG oligonucleotide comprising the nucleotide sequence of SEQ ID NO: 200 in combination with a *Bacillus anthracis* vaccine also results in unexpectedly superior results compared to administration of a *Bacillus anthracis* vaccine with another type of adjuvant.

For example, Little *et al.* (*Vaccine* 25:2771-2777, 2007, a copy of which is provided herewith) evaluate the production of anti-PA IgG antibodies in animals immunized with recombinant protective antigen (rPA) vaccine without adjuvant or with an aluminum hydroxide adjuvant. As shown in Table 2a, ten weeks after inoculation, serum antibody titers were approximately 5-fold higher in animals receiving the adjuvant, relative to vaccine alone (titers of 158 and 31.8, respectively, as measured by ELISA). Similar differences in antibody titer were observed at weeks 2, 4, 6 and 8. In contrast, administration of CpG 7909 (SEQ ID NO: 200) in combination with the AVA vaccine results in a 17-fold increase in anti-PA titer.

The combination of CpG oligonucleotide (SEQ ID NO: 200) and a *Bacillus anthracis* vaccine also results in unexpectedly superior results compared with administration of the same oligonucleotide in combination with other vaccines. For example, Jones *et al.* teach administration of an oligonucleotide with the nucleotide sequence of SEQ ID NO: 200 in combination with a peptide vaccine for malaria. As shown in Figures 1 and 2, co-administration of the CpG oligonucleotide resulted in an increase in the titer of serum antibodies specific for *Plasmodium falciparum* protein, relative to administration of a negative control oligonucleotide. However, the increase in antibody titer was only about 2-fold (by immunofluorescent antibody test, Figure 2) or 3-fold (by ELISA, Figure 1). In contrast, administration of ODN 7909 (SEQ ID NO: 200) in combination with the AVA vaccine resulted in a 17-fold increase in anthrax-specific neutralizing antibody, and passive transfer of these neutralizing antibodies conferred significantly greater protection to challenged mice, compared with mice that received antibodies elicited in response to AVA alone. Jones *et al.* did not evaluate survival following malaria challenge or protection provided by passive transfer of neutralizing antibodies. Thus, co-administration of a CpG oligonucleotide comprising the nucleotide sequence of SEQ ID NO: 200 and a *Bacillus anthracis* vaccine results in unexpectedly superior results, as evidenced by an increase in neutralizing antibodies and an increase in survival following challenge.

Applicants further submit that the significant enhancement of the immune response following vaccination with AVA conferred by the K-type oligonucleotide of SEQ ID NO: 200 was unexpected. Provided herewith is a Declaration under 37 C.F.R. §1.132 by Dr. Dennis M. Klinman, a named inventor in the application. In paragraph 5 of the Declaration, Dr. Klinman summarizes the results of studies performed in his laboratory using a heat killed influenza virus vaccine in combination with either a known immunostimulatory adjuvant, monophosphoryl lipid

A (MPL), or a K-type CpG oligonucleotide. These studies demonstrated that while MPL was an effective adjuvant, the K-type CpG oligonucleotide did not increase anti-influenza virus antibody titer or increase survival. Dr. Klinman also discusses (in paragraph 6 of the Declaration) the data described in Verthelyi *et al.*, a publication describing work from his laboratory. In particular, Verthelyi *et al.* report the finding that a K-type CpG oligonucleotide did not significantly alter the immune response when used as an adjuvant for a heat killed *Leishmania* vaccine which, like AVA, is a complex, multi-component vaccine. Given these findings, it would not have been expected that a K-type CpG oligonucleotide, including CpG 7909 (SEQ ID NO: 200), would effectively enhance the immunogenicity of a vaccine against *Bacillus anthracis*, such as AVA.

In summary, the claimed methods not only result in unexpectedly superior results over the combination of cited references, but the beneficial effects of combining a K-type CpG oligonucleotide comprising the nucleotide sequence of SEQ ID NO: 200 and a *Bacillus anthracis* vaccine would not have been predictable or expected based on the teachings of the prior art. Accordingly, Applicants request withdrawal of this rejection under 35 U.S.C. § 103(a).

Conclusion

Applicant believes that the foregoing comprises a full and complete response to the Office Action of record. Withdrawal of the pending rejections and allowance of the claims is respectfully requested. If the Examiner believes that there are any remaining issues in the case that could be resolved by a telephonic interview, the Examiner is encouraged to contact the representative for Applicant listed below to discuss any outstanding matters.

Respectfully submitted,

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